(C1)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/003,472 | 11/15/2001 | Steven A. Sundberg | 01-054310US | 4346 |

22798 7590 07/12/2004

QUINE INTELLECTUAL PROPERTY LAW GROUP, P.C.
P O BOX 458
ALAMEDA, CA 94501

| EXAMINER | |
|---|---|
| LUM, LEON YUN BON | |
| **ART UNIT** | **PAPER NUMBER** |
| 1641 | |

DATE MAILED: 07/12/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/003,472 | SUNDBERG ET AL. | |
| | Examiner | Art Unit | |
| | Leon Y Lum | 1641 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *15 November 2001*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-108* is/are pending in the application.

4a) Of the above claim(s) *9,14-15,18,19,22,23,28 and 76-108* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-13,16,17,20,21,24-27 and 29-75* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-108* are subject to restriction and/or election requirement.

**Application Papers**

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 November 2001* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

### *Election/Restrictions*

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-75, drawn to a method of generating a thermal property curve, classified in class 436, subclass 172.

II. Claims 76-108, drawn to an integrated system or microfluidic device, classified in class 422, subclass 68.1.

1. The inventions are distinct, each from the other because of the following reasons:

2. Inventions I and II are related as process and apparatus for its practice. The inventions are distinct if it can be shown that either: (1) the process as claimed can be practiced by another materially different apparatus or by hand, or (2) the apparatus as claimed can be used to practice another and materially different process. (MPEP § 806.05(e)). In this case the product can be used to practice the materially difference processes of fluid separation and molecule detection.

3. Because these inventions are distinct for the reasons given above and the search required for Group I is not required for Group II, restriction for examination purposes as indicated is proper.

4. In the event that Applicant elects Group I the following species election must also be made:

a. Elect one "transporting" method

i. Simultaneously transporting, claim 8

ii. Sequentially transporting, claim 9

b. Elect one "selected period of time"

i. 0.1 second to about 1.0 second, claims 14 and 18

ii. 0.1 second to about 10 seconds, claims 15 and 19

iii. 0.1 second to about 1.0 minute, claims 16 and 20

c. Elect one "temperature"

i. 10 °C to about 60 °C, claim 22

ii. 10 °C to about 90 °C, claim 23

iii. 10 °C to about 100 °C, claim 24

d. Elect one "joule heating"

i. Joule heating occurs over the entire length, claim 27

ii. Joule heating occurs over a selected zone, claim 28

5. Currently, claims 1-7, 10-13, 17, 21, 25-26, and 29-75 are generic. Claims 8-9, 14-16, 18-20, 22-24, and 27-28 are subject to species election.

6. For example, if Applicant elects Group I and species a(i), b(i), c(i), and d(i), the election consists of claims 1-8, 10-14, 17-18, 21-22, 25-27, and 29-75.

7. In the event that Applicant elects Group II the following species election must also be made:

a. Elect one "joule heating"

i. Joule heating occurs over the entire length, claim 83

ii. Joule heating occurs over a selected zone, claim 84

8. Currently, claims 76-82 and 85-108 are generic. Claims 83-84 are subject to species election.

9. For example, if Applicant elects Group II and species a(ii), the election consists of claims 76-82 and 84-108.

10. During a telephone conversation with Paul Littlepage on June 29, 2004 a provisional election was made without traverse to prosecute the invention of Group 1 and species claims 8, 16, 20, 24, and 27. The elected invention consists of claims 1-8,

10-13, 16-17, 20-21, 24-27, and 29-75. Affirmation of this election must be made by applicant in replying to this Office action. Claims 9, 14-15, 18-19, 22-23, 28, and 76-108 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

11. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

***Information Disclosure Statement***

12. The foreign patents and non-patent literature listed in the information disclosure statement filed September 23, 2002 were received. However, the Examiner does not have access to the documents. A request has been sent internally to locate the documents and send them to the Examiner. Therefore, Examiner has not considered the foreign patents and non-patent literature crossed out on PTO form 1449, but will consider them when the documents have been received.

***Drawings***

13. The drawings are objected to because reference number 400, drawn to a microfluidic chip (page 19, line 12) for Figure 4a, is not shown in said figure. Corrected drawing sheets are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. The replacement sheet(s) should be labeled "Replacement Sheet" in the page header (as per 37 CFR 1.84(c)) so as not to obstruct any portion of the drawing figures. If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

***Claim Objections***

14. Claims 3, 30, 46, 50, 54-55, and 57 are objected to because of the following informalities: the term "comprises" in the instant claims refers to Markush groups and should be replaced by the phrase "consists of". Appropriate correction is required.

15. Claim 63 is objected to because of the following informalities: the term "comprising" refers to a Markush group and should be replaced by the phrase "consisting of ". Appropriate correction is required.

16. Claim 7 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 7 recites "the method further comprising heating the molecules and detecting at least one physical property of the first molecule" (lines 5-6 of the claim) and "while the molecules are heated in the microchannel or microchamber." (lines 6-8 of the claim). However, claim 1 recites "heating the at least one molecule in the at least one microchannel or microchamber" (line 4 of the claim) and "detecting at least one detectable property of the at least one molecule during the heating" (line 5 of the claim). Therefore, the "heating" and "detecting" steps have been previously recited in the parent claim with respect to "the first molecule" in the instant claims, and said steps are in improper dependent form for failing to further limit the subject matter of claim 1.

***Claim Rejections - 35 USC § 112***

17. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

18. Claims 16, 20, 24-34, 36, 47-48, and 64-65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

19. In claim 36, the phrase "wherein the detectable property comprises a change in the total free energy of a system comprising the at least one molecule in the at least one microchannel or microchamber" (lines 1-3 of the claim) is vague and confusing. The parent claim, claim 1, states "detecting at least one detectable property of the at least one molecule during the heating" (lines 5-6 of the claim), which indicates that the "detectable property" is a characteristic of the "at least one molecule". However, the phrase in the instant claim states that the "detectable property" is "the total free energy of a system", which includes much more than just the "molecule". It is not clear how the "detectable property" can be from the microfluidic device when it has been defined in claim 1 as a characteristic of the "molecule" only.

20. In claims 36, 47-48, and 64-65, the phrase "total free energy" is vague and confusing. The specification does not provide a definition for the term and it is not clear what the phrase is directed towards. With respect to claim 36, it is not clear how a "change" in "total free energy" can be determined since the phrase "total free energy" is vague and confusing. With respect to claims 47-48 and 64-65, it is not clear how "total

free energy" can discerned or measured since the phrase is does not present a clear understanding of what is to be discerned or measured.

21. In claims 16, 20, and 24-25, the term "or more" is vague and confusing. This term follows a "selected period of time" in claims 16 and 20 and a "selected temperature" in claim 24 (line 2 of the claims). However, the term "or more" alters the range set by the prior numbers. It is not clear when an appropriate "selected period of time" or "selected temperature" would be obtained since there is no upper limit recited due to the term "or more", and the term is confusing since it is not clear whether the "selected period of time" or "selected temperature" is within the range stated or higher. The term "or more" also refers to a Markush group in claim 25 (line 3). However, the term is confusing since it is not clear whether there are more methods other than "joule heating or non-joule heating" and what the additional methods are. Applicant is invited to correct the claim.

22. In claims 42, 45, and 47-48, the term "discerning" (line 2 of claims 42 and 45, and line 1 of claims 47-48) is vague and indefinite. The specification does not define the term "discerning" and one of ordinary skill in the art at the time of the invention would not know how the term modifies the limitations following the term.

23. The term "about" in claims 16, 20, and 24 is a relative term which renders the claim indefinite. The term "about" (line 2 of the claims) is not defined by the claim, the

specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is not clear when a selected period of time has been obtained since it is not clear where the limits of the period are. Would 0.01 seconds, 0.001 seconds, or 1.20 minutes be applicable? What deviation from the recited numbers does the term "about" encompass? Applicant is advised to correct the claim.

### *Claim Rejections - 35 USC § 102*

24. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

25. Claims 1-3, 5-7, 13, 16-17, 20-21, 24-27, 29-35, 37-42, 49-54, 62-63, and 66-69 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Chow et al (USP 5,965,410).

In the instant claims, Chow et al reference teaches a method of generating a thermal property curve for at least one molecule in a microfluidic device, comprising the steps of flowing the at least one molecule into at least one microchannel, whereby fluid containing nucleic acid was introduced into the microchannel (column 32, lines 52-54), heating the at least one molecule in the at least one microchannel, whereby the nucleic

acid is heated in a microchannel (column 32, lines 46-47), detecting at least one detectable property of the at least one molecule during the heating, whereby upon denaturation or melting of a molecular beacon consisting of a fluorescent donor-acceptor pair coupled to opposite ends of a self-hybridizing nucleic acid, a fluorescent reaction mixture occurs (column 33, lines 55-65) and is measured over time (column 34, lines 5-8), and generating a thermal property curve for the at least one molecule, whereby a plot is made of the fluorescence (column 34, lines 12-18 and Figure 18).

With regards to claims 2-3, Chow et al teach that the at least one molecule is a nucleic acid and also teach the step that at least one molecule changes at least one of its physical properties by denaturing in response to temperature, whereby processes for treatment of nucleic acid material occurs by successively changing temperature of the nucleic acid material by way of thermal cycling in order to effect a complete or partial change from double stranded for to single stranded form (column 30, lines 59-66).

With regards to claim 5, Chow et al teach the step wherein flowing comprises electrokinetically transporting the at least one molecule through the at least one microchannel, whereby electrokinetic material transport systems transport and direct materials within an interconnected channel through the application of electrical fields to the materials (column 8, lines 13-21).

With regards to claim 6, Chow et al teach the step wherein flowing comprises transporting the at least one molecule through the at least one microchannel under positive or negative pressure, whereby fluid transport and direction may be controlled in

whole or in part, using pressure based flow systems that incorporate external or internal pressure sources to drive fluid flow (column 10, lines 47-51).

With regards to claims 7, 37, 49, and 51-54, Chow et al teach the steps wherein flowing the at least one molecule into the at least one microchannel comprises transporting at least a first molecule through the at least one microchannel and contacting the at least first molecule in the at least one microchannel with at least a second molecule, the method further comprising heating the molecules and detecting at least one physical property of the first molecule while the molecules are heated in the microchannel and measuring a fluorescence of the first molecule in the presence of the at least second molecule as a function of temperature, whereby fluid containing nucleic acid was introduced into the microchannel (column 32, lines 52-54), an intercalating dye was added to the fluid to provide a fluorescent signal, depending upon whether the nucleic acid was double stranded or denatured (column 32, lines 56-58), detecting a change in a dielectric property of the at least one molecule, measuring the melting temperature of the at least second molecule, wherein the first molecule consists of two receptors, and wherein the second molecule consists of a nucleic acid, whereby the nucleic acid is heated in a microchannel (column 32, lines 46-47), and upon denaturation or melting of a molecular beacon consisting of a fluorescent donor-acceptor pair coupled to opposite ends of a self-hybridizing nucleic acid, a fluorescent reaction mixture occurs (column 33, lines 55-65) and is measured over time (column 34, lines 5-8). Although Chow et al do not explicitly teach contact between the at least first molecule and at least second molecule, the fluorescent signal provided from the

intercalating dye is dependent on the physical state of the nucleic acid. Intercalating dyes are also well-known to those of ordinary skill in the art at the time of the invention as dyes that function by inserting themselves into DNA. Therefore, contact between the nucleic acid and intercalating dye is necessary for providing the fluorescent signal.

With regards to claim 13, Chow et al teach the step wherein heating comprises elevating the temperature of the at least one molecule for a selected period of time, whereby a molecular beacon was placed in a microfluidic channel and heating was controlled by applying current through the channel and was first increased from about 20 °C to about 95 °C (column 33, lines 66-67 and column 34, lines 1-9).

With regards to claims 16-17 and 20, Chow et al teach that the selected period of time comprises about 0.1 second to about 1.0 minute or more, whereby current is applied to decrease intensity between 153 to 158 seconds (column 33, lines 1-6 and Figure 17). Chow et al also teach that heating comprises heating the at least one molecule at a selected point in time after contacting the at least one molecule by at least a second molecule and flowing the at least one molecule into the at least one microchannel, whereby the current applied disclosed above is performed after fluid containing the DNA and the dye was introduced into the channel of the substrate in the microfluidic system (column 32, lines 66-67 and column 33, line 1).

With regards to claims 21 and 24, Chow et al teach the step wherein heating comprises elevating the temperature of the at least one molecule to a selected temperature of about 10 °C to about 100 °C, whereby strand separation require the use

of high temperatures of at least 60 °Celcius and often 100 °Celcius or more (column 31, lines 12-13).

With regards to claims 25-27 and 30-31, Chow et al teach the steps of elevating the temperature of the at least one molecule in the at least one microchannel by joule heating, flowing a selectable electric current through the at least one microchannel, joule heating occurs over the entire length of the at least one microchannel, and controlling the selectable current comprising direct or alternating current, whereby power is applied to fluid in channel 705 and 709 with energy in the form of electric current , and the electric current can be direct and alternating (column 18, lines 13-24 and Figure 5).

With regards to claim 29, Chow et al teach flowing a selectable electric current through at least a first section of the at least one microchannel with a first cross-section and through at least a second section of the at least one microchannel with a second cross-section, and the first cross-section is greater in size than the second cross-section, thereby causing the second cross-section to have a higher electrical resistance than the first cross-section, thereby further causing the second cross-section to have a higher temperature than the first cross-section, whereby regions 505 and 513 that attach, respectively, to regions 501 and 503, has a smaller cross-section dimension than outer portions 507 and 511, and the narrower dimensions result in an increased current density within these regions when a current is passed through channel 502, resulting in a heating of the fluid located within these regions (column 17, lines 8-26 and Figure 3).

With regards to claims 32-34, Chow et al teach the steps of non-joule heating comprising elevating the temperature of the at least one microchannel through use of an external heat source comprising a thermal heating block and the non-joule heating occurs over the entire length of the at least one microchannel, whereby external resistive heating coils or material provide heat to the fluidic system in a conductive manner, and provides a uniform temperature distribution to be present on the chip (column 2, lines 1-8).

With respect to claims 35, 38-42, 50, 62-63, 66-69, Chow et al teach that the detectable property comprises fluorescence and that detecting comprises the at least one molecule comprises a first molecule and a second molecules, whereby optical detection systems include systems that are capable of producing light at an appropriate wavelength for activating the fluorescent material (column 12, lines 13-16), measuring the light emitted from material within the channel, the transmissibility of the material, and an amount of light emitted from the material, such as fluorescent material (column 11, lines 57-64), wherein the first molecule comprises a fluorescence indicator dye, wherein the at least second molecule consists of two or more of a nucleic acid, whereby a molecular beacon consisting of a fluorescent donor-acceptor pair is coupled to opposite ends of a self-hybridizing nucleic acid sequence (column 33, lines 57-59), a change in applied electric current needed to maintain a constant temperature of the at least one molecule in the at least one microchannel with and without the presence of the second molecule, and measuring a change in a dielectric property of the first molecule as a function of temperature when the first molecules is in the presence and

without the presence of the at least second molecule, whereby the temperature of fluid is controlled by adjusting a first parameter from the electrical source (column 4, lines 30-37), with application for separation of complementary strands of a nucleic acid (column 31, lines 29-34) and denaturation with an intercalating dye (column 32, lines 56-59).

***Claim Rejections - 35 USC § 103***

26. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

27. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

28. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

29. Claims 4, 8, and 10-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Nikiforov et al (USP 5,964,995).

Chow et al reference has been disclosed above, but fail to disclose that the at least one molecule comprises a complex of two or more molecules; and wherein the complex comprises at least a first enzyme and at least a substrate, and wherein flowing comprises simultaneously transporting the at least first molecule and the at least second molecule through the at least one microchannel, wherein the at least microchannel comprises at least a first microchannel and at least a second microchannel, and wherein the at least first molecule is transported through the at least first microchannel and the at least second molecule is transported through the at least second microchannel.

Nikiforov et al teach an assay in a microfluidic device having a channel structure with currents placed in the chip (column 12, lines 36-67 and column 13, lines 1-21) and utilizing electroosmotic flow of fluids (column 2, lines 60-63), wherein an injection cycle was performed with a reagent concentration of substrate+enzyme by injecting them into an injection channel (main reaction channel) from separate wells (column 13, lines 7-21

and Figure 3), wherein the substrate is dFMUP and the enzyme is LAR (column 12, lines 55-58), in order to determine the inhibition of LAR enzyme in the presence and absence of NDSB.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Chow et al by including a substrate+enzyme molecule, as taught by Nikiforov et al, in order to perform an assay in a microfluidic system to determine the inhibition of LAR enzyme in the presence and absence of NDSB. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including the substrate+enzyme molecule, as taught by Nikiforov et al, in the method of Chow et al, since Chow et al teach the steps of flowing, heating, and detecting at least one molecule in a microfluidic system with electrokinetic transportation, and the substrate+enzyme molecule has been taught to be used in said steps in a microfluidic system with electroosmotic flow, which is a type of electrokinetic transportation.

30. Claims 36, 47-48, and 64-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Gonzales et al (The Journal of Biological Chemistry, 272(17), 1997.

Chow et al reference has been disclosed above, but fails to disclose that the detectable property comprises measuring a change in the total free energy of a system comprising the at least one molecule in the at least one microchannel, with and without the presence of the second molecule.

Gonzales et al reference teaches protein denaturation of biotin-streptavidin complexes by differential scanning calorimetry to produce thermograms and obtain the change in enthalpy (ΔH) for ligands of various saturations, including the absence of ligand or under conditions of partial or full ligand saturation, in order to explore protein thermostability and study protein-ligand interactions (page 11288, right column, first to sixth paragraphs and Figure 1).

It would have been obvious to modify the method of Chow et al by measuring a change in enthalpy (ΔH) for ligands of various saturations, as taught by Gonzales et al, in order to explore protein thermostability and study protein-ligand interactions. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including the step of measuring a change in enthalpy (ΔH) for ligands of various saturations, as taught by Gonzales et al, since Chow et al teach a method to determine the thermal property curve of a molecule, and differential scanning calorimetry to produce enthalpy (ΔH) is one method of generating a thermal property curve.

31. Claims 43 and 55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Regnier et al (USP 5,958,202).

Chow et al reference has been disclosed above, but fail to disclose that detecting and generating the thermal property curve comprises that the at least one molecule comprises a second molecule and a first molecule that is a protein.

Regnier et al reference teach protein samples that may be injected into a capillary electrophoresis apparatus (column 39, lines 45-46 and Figure 8), that the protein is a competitor (column 7, lines 60-61), and that the competitor will react with a first reactant (column 8, lines 1-6), wherein the first reactant is an enzyme substrate (column 7, line 29), in order to perform analysis of analytes, wherein the analytes are products detectable to include any substance which possess the property of fluorescence (column 5, lines 20-35).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Chow et al by including protein samples, as taught by Regneir et al, in order to perform analysis of analytes, wherein the analytes are products detectable to include any substance which possess the property of fluorescence. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including the protein samples, as taught by Regnier et al, in the method of Chow et al, since Chow et al teach the steps of flowing, heating, and detecting at least one molecule in a microfluidic system, wherein the detection includes detecting fluorescence, and the protein molecule has been taught to have fluorescent properties and be able to function in a capillary electrophoresis microfluidic system.

32. Claims 44-45 and 56-61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Pantoliano et al (USP 6,020,141).

Chow et al reference has been disclosed above, but fail to disclose that the first molecule further comprises one or more tryptophan residues, that the at least second molecule is a ligand, the step of exciting one or more tryptophan residues, thereby creating excited tryprophan residues, the step of discerning an emission of the one or more excited tryptophan residues, measuring a fluorescence of the one or more excited tryptophan residues, in the presence of and without the presence of the at least second molecule, as a function of temperature, the step of measuring the melting point of the at least one molecule, and wherein a change in the fluorescence of tryptophan residues is proportional to a change in the physical property of the at least first molecule due to a change in temperature.

Pantoliano et al reference discloses a microplate thermal shift assay of human α-thrombin (first molecule) with ligand binding (second molecule) and temperature heating at 44 °C and 64 °C (column 51, lines 16-67; column 52, lines 1-8; and Figure 3), a microplate thermal shift assay wherein intrinsic Trp (Tryptophan) fluorescence of human α-thrombin (first molecule) was assayed, wherein the samples were exposed to light at 280 nm and emission was detected at 350 nm, with temperature cycling performed, and observation of a midpoint temperature $T_m$ at 334.4±5.1 °K (column 53, lines 55-67; column 54, lines 1-12; and Figures 13-14), in order to determine a change in a biophysical parameter of a protein as a function of increasing temperature (column 3, lines 12-14).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Chow et al by including Tryptophan residues in a

protein sample and the additional limitations disclosed above, as taught by Pantoliano et al, in order to determine a change in a biophysical parameter of a protein as a function of increasing temperature. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including the Tryptophan residues in a protein sample and other limitations disclosed above, as taught by Pantoliano, in the method of Chow et al, since Chow et al teach thermal assays to produce thermal property curves, and the sample with Tryptophan residue has been disclosed as able to produce a thermal profile in an assay with temperature cycling.

33. Claims 46, 70-71, and 73-75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Clegg et al (Proceedings of the National Academy of Sciences, 90, 1993).

Chow et al reference has been disclosed above, but fails to disclose the step of determining a peak temperature in the microfluidic device through construction of a thermal property curve for one or more molecules of known $T_m$, that the molecule comprises a first molecule and at least a second molecule, wherein the first molecule and at least second molecule bind to each other over a known temperature range, wherein the first molecule and the at least second molecule together comprise a complementary double-stranded nucleic acid molecule of known sequence and known $T_m$, wherein the first molecule and the at least second molecule are each labeled with an indicator molecule, wherein the first molecule and the at least second molecule are each labeled with a different indicator molecule, thus allowing detection of a separation

of the first molecule and the at least second molecule, and that detecting comprises use of fluorescence resonance energy transfer.

Clegg et al reference teaches $T_m$ measurements of DNA samples (page 2995, left column, second paragraph, and Figure 3) which are duplex molecules of complimentary oligonucleotides of known sequences, each strand labeled with either fluorescein or rhodamine (page 2994, right column, third paragraph) so that FRET (fluorescence resonance energy transfer) measurements can be taken after melting of the duplex structures (page 2995, left column, fourth paragraph), in order to investigate structural and conformational problems with nucleic acids (page 2997, right column, third paragraph) using a method that is accurate, reproducible, and self-normalizing (page 2998, left column, third paragraph).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Chow et al, by including the steps and limitations disclosed above, as taught by Clegg et al, in order to investigate structural and conformational problems with nucleic acids using a method that is accurate, reproducible, and self-normalizing. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including the steps taught by Clegg et al in the method of Chow et al, since Chow et al teach temperature induced denaturing of complimentary nucleic acid strands to produce thermal property curves through fluorescence emissions, and the steps and limitations disclosed above, as taught by Clegg et al, are an example of denaturing of complimentary strands with fluorescent detection.

34. Claim 72 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chow et al (USP 5,965,410) in view of Clegg et al (Proceedings of the National Academy of Sciences, 90, 1993) as applied to claim 71 above, and further in view of Gonzales et al (The Journal of Biological Chemistry, 272(17), 1997.

Chow et al and Clegg et al references have been disclosed above, but fail to disclose that the first molecule and the at least second molecule are biotin and streptavidin.

Gonzales et al reference teaches biotin and streptavidin as protein and ligand in order to explore protein thermostability and study protein-ligand interactions (page 11288, left column, third paragraph and right column, first to third paragraphs).

It would have been obvious to modify the methods of Chow et al and Clegg et al with biotin and streptavidin for biotin-streptavidin binding, as taught by Gonzales et al, in order to explore protein thermostability and studying protein-ligand interactions. One of ordinary skill in the art at the time of the invention would have reasonable expectation of success in including biotin and streptavidin, as taught by Gonzales et al, in the methods of Chow et al and Clegg et al, since Chow et al and Clegg et al teach protein samples and temperature denaturation of samples, and biotin-streptavidin complex is an example of a complexed protein that can undergo protein denaturation.

***Conclusion***

35. The prior art made of record and not relied upon is considered pertinent to Applicant's disclosure.

Southgate et al (US 5,863,502) teach a microfluidic device with DNA denaturing and heating by electrical current.

Dubrow et al (US 5,976,336) teach electrophoretic transportation of samples in microfluidic devices and electrical current applied to sections along current flow.

Hollis et al (US 5,653,939) teach microfluidic devices with heat-sensitive array detectors and resistors heated by electrical current.

36. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Leon Y Lum whose telephone number is (571) 272-2878. The examiner can normally be reached on 8:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

LYL

Leon Lum
Patent Examiner
AU 1641

LONG V. LE
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600
07/08/07